Effect of neem compared with *Pseudomonas fluorescens* on the management of cowpea root rot disease

Vengadeshkumar, L*., Kalaiselvi, M., Meera, T., Sanjayghandi, S., Udhayakumar, R., Rajamohan, K. and Sudhasha, S.

ABSTRACT

Cowpea also known as 'black eye beans' (*Vigna ungiculata L.*) is an important leguminous and hay crop in tropical and subtropical regions. In recent years root rot caused by *Macrophomina phaseolina* causes significant losses in cowpea. Hence, the present study was undertaken to assess the bio-potential of neem cake with *Pseudomonas fluorescens* on the management of cowpea root rot disease. In this study, *P. fluorescens* as seed and soil application (@ 10 gm/kg and 2.5 kg/ha) with neem oil cake as soil application @ 0.25 t/ha (T_6) significantly reduced the root rot disease incidence with a maximum per cent disease reduction and increased the biometrics of cowpea plant under both pot and field trials.

Keywords: *Macrophomina phaseolina,* cowpea, neem, *in-vivo*.

MS History: 13.03.2019 (Received)-20.05.2019 (Revised)- 15.12.2019 (Accepted).

Citation: Vengadeshkumar, L*., Kalaiselvi, M., Meera, T., Sanjayghandi, S., Udhayakumar, R., Rajamohan, K. and Sudhasha, S. 2019. Effect of neem compared with *Pseudomonas fluorescens* on the management of cowpea root rot disease. *Journal of Biopesticides,* **12**(2):232-238.

INTRODUCTION

Cowpea also known as 'black eye beans'(*Vigna ungiculata L.*) is an important leguminous and hay crop in tropical and subtropical regions, especially in the dry savanna region of West Africa (Fang *et al*., 2007). Cowpea originated in Africa and South East Asia and in the southern United States (Fatokum *et al*., 2000; Shaw, 2007). Nigeria is the world's largest producer and consumer of cowpea, as it produces over 2.7 Million Tonnes of cowpea annually with an average yield of 417 kg/ha. It produces both white and brown varieties (FAO, 2017). Cowpea is affected by many diseases caused by viruses, bacteria, fungi and nematodes (Emechebe and Lagoke, 2002). Among the diseases, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is an important fungal disease that significantly reduces growth and yield in arid regions of the world (Marroni, 2015). Biological control of plant pathogens is a potential non-chemical means and is known to be a cheap and effective eco-friendly method for the management of crop diseases (Harman, 1991). Among the biological methods, the use of plant growth-promoting rhizobacteria (PGPR) would be an attractive alternative to decrease the use of chemical fungicide which also effect environmental pollution (Ali *et al.,* 2010). In the context of the international concern for food and environmental quality, PGPR's have been applied to various crops to suppress pathogens, enhance seedling emergence, crop growth and yield (Nayaka *et al.,* 2009; Minaxi and Saxena, 2010 and Misk and Franco, 2011). Besides the use of PGPR's against pathogen the antifungal activity of various plant products *viz*., cotton cake (Ehteshamul-Haque *et al.,* 1995), pungam cake (Karthikeyan *et al.,* 2006), mustard cake and cotton cake (Anis *et al.,* 2010) and neem cake (Dhingani *et al.,* 2013; Meena *et al*., 2014) and onion bulb extract (Tandel *et al.,* 2010; Muzammil *et al.,* 2014; Savaliya *et al.,* 2015) against *M. phaseolina* causing root rot of various crops has also been well documented. In this study, assessment of the effect due to treatment with *P. fluorescens* and neem cake on root rot incidence and the

biometrics of cowpea in *in-vivo* condition has been done.

MATERIALS AND METHODS

Preparation of solid formulation of biocontrol agents

A loopful of *P. fluorescens* isolates was inoculated into the sterilized King's B broth and incubated in a rotary shaker at 150 rpm for 72 h. at room temperature (28 ± 2°C). After 72 h, 400 ml of bacterial suspension containing 9×10^8 cfu ml^{-1}, one kg of the carrier material (talc powder), 15 g calcium carbonate and 10 g CMC were thoroughly mixed, shade dried to reduce the moisture content below twenty per cent and packed in polythene bags (Vidhyasekaran *et al.*, 1996).

Preparation of aqueous extracts

The freshly collected oil cake was separately ground and 40gm of the ground powder was suspended in 150 ml sterilized distilled water in a conical flask and kept aside 15 days. The flasks were shaken every day for thorough mixing and dissolution of the content. After 15 days, the extracts were first passed through four layers of muslin cloth and then filtered through Whatman No: 41 filter paper into 150 ml conical flasks and tightly wrapped with aluminium foil and autoclaved at 121ºC at 15 psi pressure for 20 minutes (Dhingani *et al.*, 2013).

Effect of *P. fluorescens* and neem cake on the management of cowpea root rot disease (pot culture)

Sterilized soil (1.0 kg) was mixed with the pathogen inoculums @ five per cent level (multiplied on sand maize medium) and filled in 15 x 30 cm dia. earthen pots. Solid formulation of the antagonist was applied to the soil 10 days before sowing as per the treatment schedule. In addition to the organic amendment neem cake is added to the treatments in order to provide a food base for the antagonists applied through dual delivery systems. The treatment schedule followed is mentioned below: T_1: Seed treatment with *P. fluorescens* @ 10 gm/kg of seeds; T_2: Soil application with *P. fluorescens* @ 2.5 kg/ha; T_3: T_1+T_2; T_4: Soil application with Neem cake @ 0.25 t/ha; T_5: T_2 + T_4; T_6: T_3 + T_4, T_7: Seed treatment and soil drenching of Carbendazim 50 WP 2g/kg and 0.1%) and T_8: control.

The experiment was conducted in a randomized block design and replicated thrice. Soil drenching with carbendazim @ 0.1% and seed treatment with carbendazim @ 2 g/kg of seeds was used for comparison and pathogen alone inoculated pots served as control. The treated seeds were sown in pathogen inoculated soil @ 5 seeds per pot and maintained with need based irrigation following all standard agronomic practices. The observations on the incidence of dry root rot disease (%) (Assessed at 30, 60 and 90 DAS), germination (% - assessed at 10 DAS), biomass, number of pods per plant, seed yield per pot and 100 seed weight were recorded at the time of harvesting following standard procedures.

Management of cowpea root rot disease-Field trial

Based on the best results obtained from the pot culture experiments, a field trial under rainfed condition was conducted in a farmer's field. The field experiment was laid out in a randomized block design with eight treatments and three replications in a plot size 5×4 sq.m per treatment. The treatment schedule followed is mentioned below: T_1 : Seed treatment with *P. fluorescens* @ 10 gm/kg of seeds; T_2 : Soil application with *P. fluorescens* @2.5 kg/ha; T_3 : T_1+T_2; T_4 : Soil application with Neem cake @0.25 t/ha; T_5 : T_2 + T_4 T_6 : T_3 + T_4; T_7 : Seed treatment of Carbendazim 50 WP 2g/kg and T_8 : control. The treatments were given as per the schedule and all the agronomic practices were followed as per standard procedures as recommended by the State Agricultural Department. The observations on the germination (% - assessed at 10 DAS), root length (cm) and shoot length (cm) incidence of dry root rot disease (%) (Assessed at 30, 60 and 90 DAS) and yield kg/ha of cowpea crop was recorded at the time of harvesting following standard procedures.

RESULT AND DISCUSSION

Root rot incidence of cowpea-pot culture

Results depicted in table 1 showed that the T_6 treatment with *P. fluorescens* as seed and soil application (@ 10 gm/kg of seed and 2.5 kg/ha) plus neem oil cake as soil application (@ 0.25 t/ha) controlled the root rot disease incidence (6.54%) significantly with 70.97 per cent of disease reduction over control. It was followed by T_3 (Seed treatment with *P. fluorescens* @ 10 gm/kg plus soil application with *P. fluorescens* @ 2.5 kg/ha) and T_5 (soil application with *P. fluorescens* @ 2.5 kg/ha plus soil application with neem oil cake @ 0.25 t/ha) with 7.95 and 9.94 per cent disease incidence respectively.

Table 1. Effect of *P. fluorescens* plus neem cake on the root rot incidence of cowpea (Pot culture)

Tr .No.	Treatments	Root rot incidence (%) 30 DAS	60 DAS	90 DAS	Mean	Per cent decrease over control
T_1	Seed treatment with *P. fluorescens* @10 gm/kg	12.76^{e}	14.88^{e}	17.72^{e}	15.12	32.88
T_2	Soil application with *P. fluorescens* @ 2.5 kg/ha	10.65^{d}	12.84^{d}	14.37^{d}	12.62	43.98
T_3	T_1+ T_2	5.44^{b}	7.82^{b}	10.61^{b}	7.95	64.71
T_4	Soil application with neem cake @ 0.25 t/ha	13.83^{f}	15.14^{f}	18.84^{f}	15.93	29.29
T_5	T_2+ T_4	7.27^{c}	9.74^{c}	12.82^{c}	9.94	55.88
T_6	T_3+T_4	4.67^{a}	6.32^{a}	8.53^{a}	6.54	70.97
T_7	Carbendazim 50 WP @ 0.1% as ST 2g/kg	4.23^{a}	6.14^{a}	8.22^{a}	6.19	72.52
T_8	Control	17.41^{g}	21.73^{g}	28.45^{g}	22.53	-

The test fungicide Carbendazim 50% WP (0.1%) as soil application recorded 6.19 per cent root rot incidence which accounting for 72.52 per cent disease reduction. In the present study the treatment with *P. fluorescens* alone as seed treatment and soil application showed significant reduction in the root rot incidence and enhanced the plant growth and yield of cowpea. The efficacy of *P. fluorescens* against various plant pathogens was reported by several workers (Gnanamanickam *et al.,* 1992; Vidhyasekaran *et al.,* 1996). Application of *P. fluorescens* isolates CTPf31 significantly reduced the root rot incidence of safflower under green house condition (Govindappa *et al.,* 2011). Ushamalini *et al.* (1997) reported that neem cake and extract of *V. negundo* effectively reduced root rot disease incidence. Also, sunflower cake followed by neem cake provided significantly reduced incidence of cowpea root rot (Bharadwaj and Sahu. 2015).

Root rot incidence and biometrics of cowpea-Field trial

Results depicted in table 3 showed that the treatment T_6 with *P. fluorescens* as seed and soil application (@ 10 gm/kg of seed and 2.5 kg/ha) plus neem cake as soil application (@0.25 t/ha) controlled the root rot disease incidence (9.81%), with maximum per cent disease reduction (64.40%).Generally, all the treatments significantly increased the biomass when compared to carbendazim 50% WP treatment and control.

The untreated control recorded the minimum biomass and number of pods per plant. Regarding seed yield and 100 seed weight, all the treatments significantly

increased the seed yield and 100 seed weight compared to carbendazim 50% WP treatment and control. The effect of different treatments on the seed yield and 100 seed weight was recorded in Table 4. Among the treatments, the maximum seed yield and 100 seed weight was observed in treatment T_6 which was better than carbendazim 50% WP and control treatments. The data on the effect of different treatments on the biomass of cowpea crop was recorded in table 4. Among the treatments, the maximum biomass and number of pods per plant (31.13gm and

Table 2. Effect of *P. fluorescens* plus neem cake on the bio metrics of cowpea (pot culture)

Tr. No.	*Treatments*	Bio mass g/ plant	No. of pods per plant	Seed yield/ pot (g)	100 seed weight (gm)	Treatments
T_1	Seed treatment with *P. fluorescens* @10 gm/kg	16.24^{e}	9.00^{e}	88.41^{e}	88.41^{e}	6.93^{e}
T_2	Soil application with *P. fluorescens* @ 2.5 kg/ha	18.22^{d}	10.00^{d}	90.46^{d}	90.46^{d}	7.24^{d}
T_3	T_1+ T_2	20.26^{b}	12.00^{b}	102.12^{b}	102.12^{b}	7.69^{b}
T_4	Soil application with neem cake @ 0.25 t/ha	15.15^{f}	8.00^{f}	74.54^{f}	74.54^{f}	6.57^{f}
T_5	T_2+ T_4	19.13^{c}	11.00^{c}	96.76^{c}	96.76^{c}	7.52^{c}
T_6	T_3+T_4	22.51^{a}	14.00^{a}	112.92^{a}	112.92^{a}	7.84^{a}
T_7	Carbendazim 50 WP @ 0.1% as ST 2 g/kg	13.14^{g}	9.00^{g}	71.51^{g}	71.51^{g}	6.31^{g}
T_8	Control	11.24^{h}	4.00^{h}	68.48^{h}	68.48^{h}	6.16^{h}

Table 3. Effect of *P. fluorescens* plus neem cake on root rot incidence of cowpea (Field trial)

Tr .No.	Treatments	Root rot incidence (%) 30 DAS	60 DAS	90 DAS	Mean	Per cent decrease over control
T_1	Seed treatment with *P. fluorescens* @10 gm/kg	14.26^{e}	20.32^{e}	28.51^{e}	21.03	22.99
T_2	Soil application with *P. fluorescens* @ 2.5 kg/ha	12.05^{d}	16.22^{d}	20.32^{d}	16.19	40.68
T_3	T_1+ T_2	9.53^{b}	11.25^{b}	13.96^{b}	11.58	57.59
T_4	Soil application with neem cake @ 0.25 t/ha	16.18^{f}	18.14^{f}	23.18^{f}	19.16	29.84
T_5	T_2+ T_4	10.38^{c}	12.08^{c}	18.72^{c}	13.72	49.76
T_6	T_3+T_4	7.86^{a}	9.38^{a}	12.20^{a}	09.81	64.40
T_7	Carbendazim 50 WP @ 0.1% as ST 2g/kg	8.50^{a}	10.75^{a}	13.71^{a}	10.98	59.79
T_8	Control	19.54^{g}	25.16^{g}	37.25^{g}	27.31	-

17.00) was observed in treatment T_6. This was followed by T_3 and T_5 treatments in the decreasing order of merit. This was followed by T_3 and T_5 in the decreasing order of merit. Sulieman and Emuas (2009) reported that soil drenching with ginger crude extract provided the best control root rot disease of cowpea in the field condition. Likewise, the bulb extracts of onion and neem extract effectively reduced the incidence of root rot diseases caused by

M. phaseolina under field condition (Rahiman *et al.,* 2013; Parveen *et al.,* 2014; Savaliya *et al.,* 2015). All these earlier reports are in line and lend support to the present findings. Thus, the results of the present study have clearly revealed that integration of *P. fluorescens* along with oil cake like neem would have exerted a synergism and also different mechanisms of disease control which certainly enhanced greater disease suppression, enhanced plant growth and yield of cowpea and improved the consistency of biological control under varied climatic conditions.

Table 4. Effect of *P. fluorescens* plus neem cake on the bio metrics of cowpea (Field trial)

Tr. No.	*Treatments*	Bio mass g/ plant	No. of pods per plant	Seed yield/ pot (g)	*100 seed weight (gm)*
T_1	Seed treatment with *P. fluorescens* @10 gm/kg	21.27^e	12.00^e	844.67^e	8.16^e
T_2	Soil application with *P. fluorescens* @ 2.5 kg/ha	22.75^d	13.00^d	868.34^d	8.43^d
T_3	T_1+ T_2	29.25^b	15.00^b	912.43^b	9.85^b
T_4	Soil application with neem cake @ 0.25 t/ha	20.08^f	12.00^f	838.36^f	7.63^f
T_5	T_2+ T_4	28.12^c	14.00^c	897.54^c	9.16^c
T_6	T_3+T_4	31.13^a	17.00^a	940.71^a	11.46^a
T_7	Carbendazim 50 WP @ 0.1% as ST 2 g/kg	18.12^g	12.00^g	746.54^g	6.94^g
T_8	Control	17.79^h	09.00^h	567.37^h	6.13^h

REFERENCES

Ali, A., Khan, M. H., Bano, R., Rashid, H., Raja, N. I. and Chaudhry, Z. 2010. Screening of Pakistani rice (*Oryza sativa*) cultivars against *Xanthomonas oryzae* pv. *oryzae*. *Pakistan Journal of Botany,* **41**(5): 2595-2604.

Anis, M., Zaki, M. J. and Dawar, S. 2010. Effect of oilseed cakes alone or in combination with *Trichoderma* species for the control of Charcoal rot of sunflower (*Helianthus annus* l.). *Pakistan Journal of Botany*, **42**(6): 4329-4333.

Bharadwaj, N. and Sahu, R. K. 2015. Evaluation of some fungicides, botanicals and essential oils against the fungus *Colletotrichum falcatum* causing red rot of sugarcane. *The Bioscan*, **9**(1): 175-178.

Dhingani, J. C., Solanky, K. U. and Kansara, S. S. 2013. Management of root rot disease caused by *Macrophomina phaseolina* (Tassi.) Goid of chickpea through botanicals and oil cakes. *The Bioscan*, **8**(3): 739-742.

Ehtashamul-Haque, S., Abid, M. and Ghaffar, A. 1995. Efficacy of *Brady rhizobium* spp. and *Paecilomyces lilacinus* with oil cakes in the control of root rot of mung bean. *Tropical Science*, **35**: 294-299.

Emechebe, A. M. and Lagoke, S. T. O. 2002. Recent advances in research of cowpea disease. In Challenges and opportunities for enhancing sustainable cowpea production edited by Fatokun C. A., Tarawali, S. A. Singh, B. B., Komawa, P. M. and Tamo. M. 94-123.

Fang, J., Chao, C. C. C., Roberts, P. A. and Elhers, J. 2007. Genetic diversity of cowpea (*Vigna unguiculata* (L.)Walp.) in four West African and USA breeding programs as determined by AFLP analysis. *Genetic Resources and Crop Evolution*, **54**(6): 119-209.

Fatokum, C. A., Tarawali, S. A., Singh, B. B., Kormawa, P. M. and Tamo, M. 2000.

Challenges and opportunity for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference iii held at International Institute of Tropical Agriculure (IITA), Ibadan, Nigeria, 4-8 September 2000. IITA Ibadan Nigeria of the world cowpea conference III held at IITA Ibadan, Nigeria 4-8 September 2000**,** 214-220 **PP**.

Gnanamanickam, S. S., Candole, B. L. and Mew, T. W. 1992. Influence of soil factors and culture practice on biological control of sheath blight of rice with antagonistic bacteria. *Pl. and Soil,* **122**: 318-326.

Govindappa, M., Ravishankar, V. and Lokesh, S. 2011. *In vitro* and *In vivo* response of different treating agents against wilt disease of safflower. *J. of Cereals and Oilseeds,* **2**(1):16-25.

Harman, G. E. 1991. Seed treatments for biological control of plant diseases. *Crop Prot,* **10**: 166-171.

Karthikeyan, V., Sankaralingam, A. and Nakkeeran. S. 2006. Management of groundnut root rot with biocontrol agents and organic amendments. *Archives of Phytopathology and Plant Protection,* **39**(3): 215 – 223.

Meena, P. N., Tripathi, A. N., Gotyal, B. S. and Satpathy, S. 2014. Bio-efficacy of phyto-extracts and oil cakes on *Macrophomina phaseolina* (Tassi) causing stem rot disease of jute, *Corchorus spp. Journal of Appied and Natural Science,* **6**(2): 530-533.

journal of Biotechnology, **8**(16): 3806-3808.

Minaxi, K. and Saxena, J. 2010. Disease suppression and crop improvement in moong beans (*Vigna radiata*) through *Pseudomonas* and *Burkholderia* strains isolated from semi arid region of Rajasthan, Indian journal of *Biological Control*, **55**: 799–810.

Misk, A. and Franco, C. 2011. Biocontrol of chickpea root rot using endophytic action bacteria. *Biological control*, **56**: 811-822.

Muzammil, H., Muhammad, U. G., Muhammad, I. H., Muhammad, A. Z., Touseef, H., Mubashar, R. and Ulhaq, A. 2014. *In vitro* evaluation of fungicides and plant extracts for suppressing mycelium growth of *Macrophomina phaseolina* causes charcoal rot of sunflower, *International Journal of Agricultural Science,* **6**(2): 136-140.

Nayaka, D., Shanti, M. L., Bose, L. K., Singh, U. D. and Nayak, P. 2009. Pathogenicity association in *Xanthomonas oryzae* pv *oryzae*. The causal organism of bacterial leaf blight of rice ARPN. *Journal of Agricultural and Biological Science,* **3**: 12-27.

Parveen, S., Wani, A. H., Ganie, A. A., Pala, S. A. and Mir, R. A. 2014. Antifungal activity of some plant extracts on some pathogenic fungi. *Archives of Phytopathology and Plant Protection,* **47:**279-284.

Rahiman, F. A., Mahmad, N., Taha, R.M., Elias, H. and Zaman, F. H. 2013. Antimicrobial properties of *Lawsonia inermis* syn. *Lawsonia alba in vivo* and *in vitro*. Journal of *Food and Agri*culture *Environment*, **11**:502-504.

Savaliya, V. A., Bhaliya, C. M., Marviya, P. B. and Akbari, L. F. 2015. Evaluation of phytoextracts against *Macrophomina phaseolina* (Tassi) Goid causing root rot of sesame. *Journal of Biopesticides.,* **8**(2): 116-119.

Suleiman, M. N. and Emua, S. A. 2009. Efficacy of four plant extracts in the control of root rot disease of cowpea (*Vigna unguiculata* [L.] Walp). *African*

Tandel, D. H., Sabalpara, A. N. and Pandya, J. R. 2010. Efficacy of Phytoextracts on *Macrophomina Phaseolina* (Tassi) Goid causing Leaf blight of greengram. *International Journal of parma and bioscience,* **2:**1-5.

Ushamalini, K., Rajappan. and Kousalya Gangadharan. 1997. Management of charcoal rot of cowpea using biocontrol agents and plant products. *Indian Phytopath,* **50** (4): 504-507.

Vidhyasekaran, P., Muthamilan, M., Rabindran, R., Sethuraman, K. and Ananthakumar, C. M. 1996. Development of a powder formulation for *Pseudomonas fluorescens* for seed, soil and foliar applications to control root and foliar pathogens. *In*: Manibhushan Rao, K. and Mahadevan, A. (eds.) Current Trends in Life Sciences, Vol. XXI, Recent trends in biocontrol of plant pathogens, Today and Tomarrow's Printers and Publishers, New Delhi, 93-96 **PP**.

Vengadeshkumar, L*., Kalaiselvi, M., Meera, T., Sanjayghandi, S., Udhayakumar, R., Rajamohan, K. and S. Sudhasha
Department of Plant Pathology,
Faculty of Agriculture, Annamalai University,
Annamalai Nagar, Chidambaram,
Tamilnadu, India-608 002.
***Corresponding author**
Email: vengadpragathi@gmail.com
Contact no: 8667806189